10/525365

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=> d his

     (FILE 'HOME' ENTERED AT 10:20:28 ON 05 OCT 2007)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 10:21:01 ON 05 OCT 2007
L1          1378 S FLUOROSCEN?
L2            38 S TRACHYPHYLLIA (W) GEOFFROYI
L3             0 S L1 AND L2
L4            22 DUP REM L2 (16 DUPLICATES REMOVED)
L5            12 S L4 AND GREEN
                 E MIYAWAKI A/AU
L6           515 S E3
                 E ANDO R/AU
L7           594 S E3
                 E KARASAWA S/AU
L8            43 S E12
                 E MIZUNO H/AU
L9          2103 S E3
L10         3191 S L6 OR L7 OR L8 OR L9
L11           14 S L2 AND L10
L12            6 DUP REM L11 (8 DUPLICATES REMOVED)

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NEWS 2	JUL 02	LMEDLINE coverage updated
NEWS 3	JUL 02	SCISEARCH enhanced with complete author names
NEWS 4	JUL 02	CHEMCATS accession numbers revised
NEWS 5	JUL 02	CA/CAplus enhanced with utility model patents from China
NEWS 6	JUL 16	CAplus enhanced with French and German abstracts
NEWS 7	JUL 18	CA/CAplus patent coverage enhanced
NEWS 8	JUL 26	USPATFULL/USPAT2 enhanced with IPC reclassification
NEWS 9	JUL 30	USGENE now available on STN
NEWS 10	AUG 06	CAS REGISTRY enhanced with new experimental property tags
NEWS 11	AUG 06	BEILSTEIN updated with new compounds
NEWS 12	AUG 06	FSTA enhanced with new thesaurus edition
NEWS 13	AUG 13	CA/CAplus enhanced with additional kind codes for granted patents
NEWS 14	AUG 20	CA/CAplus enhanced with CAS indexing in pre-1907 records
NEWS 15	AUG 27	Full-text patent databases enhanced with predefined patent family display formats from INPADOCDB
NEWS 16	AUG 27	USPATOLD now available on STN
NEWS 17	AUG 28	CAS REGISTRY enhanced with additional experimental spectral property data
NEWS 18	SEP 07	STN AnaVist, Version 2.0, now available with Derwent World Patents Index
NEWS 19	SEP 13	FORIS renamed to SOFIS
NEWS 20	SEP 13	INPADOCDB enhanced with monthly SDI frequency
NEWS 21	SEP 17	CA/CAplus enhanced with printed CA page images from 1967-1998
NEWS 22	SEP 17	CAplus coverage extended to include traditional medicine patents
NEWS 23	SEP 24	EMBASE, EMBAL, and LEMBASE reloaded with enhancements
NEWS 24	OCT 02	CA/CAplus enhanced with pre-1907 records from Chemisches Zentralblatt

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=> s fluoroscen?
L1          1378 FLUOROSCEN?

=> s trachyphyllia (w) geoffroyi
L2            38 TRACHYPHYLLIA (W) GEOFFROYI

=> s l1 and l2
L3             0 L1 AND L2

=> 'dup rem l2
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"HELP COMMANDS" at an arrow prompt (=>).

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L4             22 DUP REM L2 (16 DUPLICATES REMOVED)

=> d 1-22 ibib ab

L4   ANSWER 1 OF 22     MEDLINE on STN                    DUPLICATE 1
ACCESSION NUMBER:    2007530894     IN-PROCESS
DOCUMENT NUMBER:     PubMed ID: 17692334
TITLE:               Crystallographic Evidence for Water-assisted Photo-induced
```

Peptide Cleavage in the Stony Coral Fluorescent Protein Kaede.

AUTHOR: Hayashi Ikuko; Mizuno Hideaki; Tong Kit I; Furuta Toshiaki; Tanaka Fujie; Yoshimura Masato; Miyawaki Atsushi; Ikura Mitsuhiko

CORPORATE SOURCE: Division of Signaling Biology, Ontario Cancer Institute and Department of Medical Biophysics, 101 College St., Toronto, Ontario, M5G 1L7, Canada.

SOURCE: Journal of molecular biology, (2007 Sep 28) Vol. 372, No. 4, pp. 918-26. Electronic Publication: 2007-06-19. Journal code: 2985088R. ISSN: 0022-2836.

PUB. COUNTRY: England: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: NONMEDLINE; IN-DATA-REVIEW; IN-PROCESS; NONINDEXED; Priority Journals

ENTRY DATE: Entered STN: 12 Sep 2007
Last Updated on STN: 12 Sep 2007

AB A coral fluorescent protein from Trachyphyllia geoffroyi , Kaede, possesses a tripeptide of His62-Tyr63-Gly64, which forms a chromophore with green fluorescence. This chromophore's fluorescence turns red following UV light irradiation. We have previously shown that such photoconversion is achieved by a formal beta-elimination reaction, which results in a cleavage of the peptide bond found between the amide nitrogen and the alpha-carbon at His62. However, the stereochemical arrangement of the chromophore and the precise structural basis for this reaction mechanism previously remained unknown. Here, we report the crystal structures of the green and red form of Kaede at 1.4 A and 1.6 A resolutions, respectively. Our structures depict the cleaved peptide bond in the red form. The chromophore conformations both in the green and red forms are similar, except a well-defined water molecule in the proximity of the His62 imidazole ring in the green form. We propose a molecular mechanism for green-to-red photoconversion, which is assisted by the water molecule.

L4 ANSWER 2 OF 22 HCAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 2006:1252707 HCAPLUS

DOCUMENT NUMBER: 146:23029

TITLE: Optical microscopy with phototransformable optical labels

INVENTOR(S): Hess, Harald F.; Betzig, Robert E.

PATENT ASSIGNEE(S): Hess, Harald, F., USA; Betzig, Robert, E.

SOURCE: PCT Int. Appl., 75 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2006127692	A2	20061130	WO 2006-US19887	20060523
WO 2006127692	A3	20070426		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY,

KG, KZ, MD, RU, TJ, TM, AP, EA, EP, OA
PRIORITY APPLN. INFO.: US 2005-683337P P 20050523
US 2006-780968P P 20060310

AB First activation radiation is provided to a sample that includes phototransformable optical labels ("PTOLs") to activate a first subset of the PTOLs in the sample. First excitation radiation is provided to the first subset of PTOLs in the sample to excite at least some of the activated PTOLs, and radiation emitted from activated and excited PTOLs within the first subset of PTOLs is detecting with imaging optics. The first activation radiation is controlled such that the mean volume per activated PTOLs in the first subset is greater than or approx. equal to a diffraction-limited resolution volume ("DLRV") of the imaging optics.

L4 ANSWER 3 OF 22 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2006712529 MEDLINE
DOCUMENT NUMBER: PubMed ID: 17092037
TITLE: Competition between energy and proton transfer in ultrafast excited-state dynamics of an oligomeric fluorescent protein red Kaede.
AUTHOR: Hosoi Haruko; Mizuno Hideaki; Miyawaki Atsushi; Tahara Tahei
CORPORATE SOURCE: Molecular Spectroscopy Laboratory, RIKEN (The Institute of Physical and Chemical Research), Wako, Saitama 351-0198, Japan.
SOURCE: The journal of physical chemistry. B, (2006 Nov 16) Vol. 110, No. 45, pp. 22853-60.
Journal code: 101157530. ISSN: 1520-6106.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200701
ENTRY DATE: Entered STN: 12 Dec 2006
Last Updated on STN: 27 Jan 2007
Entered Medline: 26 Jan 2007

AB We investigated femtosecond and picosecond time-resolved fluorescence dynamics of a tetrameric fluorescent protein Kaede with a red chromophore (red Kaede) to examine a relationship between the excited-state dynamics and a quaternary structure of the fluorescent protein. Red Kaede was obtained by photoconversion from green Kaede that was cloned from a stony coral Trachyphyllia geoffroyi. In common with other typical fluorescent proteins, a chromophore of red Kaede has two protonation states, the neutral and the anionic forms in equilibrium. Time-resolved fluorescence measurements clarified that excitation of the neutral form gives the anionic excited state with a time constant of 13 ps at pH 7.5. This conversion process was attributed to fluorescence resonance energy transfer (FRET) from the photoexcited neutral form to the ground-state anionic form that is located in an adjacent subunit in the tetramer. The time-resolved fluorescence data measured at different pH revealed that excited-state proton transfer (ESPT) also occurs with a time constant of 300 ps and hence that the FRET and ESPT take place simultaneously in the fluorescent protein as competing processes. The ESPT rate in red Kaede was significantly slower than the rate in Aequorea GFP, which highly likely arises from the different hydrogen bond network around the chromophore.

L4 ANSWER 4 OF 22 MEDLINE on STN
ACCESSION NUMBER: 2007204166 MEDLINE
DOCUMENT NUMBER: PubMed ID: 17406330
TITLE: Cell tracking using a photoconvertible fluorescent protein.
AUTHOR: Hatta Kohei; Tsujii Hitomi; Omura Tomomi
CORPORATE SOURCE: Laboratory for Vertebrate Body Plan, Center for Developmental Biology, RIKEN Kobe 2-2-3 Minatojima

Minamimachi, Chuo-ku, Kobe 650-0047, Japan..
khatta@cdb.riken.jp
SOURCE: Nature protocols, (2006) Vol. 1, No. 2, pp. 960-7.
Journal code: 101284307. E-ISSN: 1750-2799.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200709
ENTRY DATE: Entered STN: 5 Apr 2007
Last Updated on STN: 22 Sep 2007
Entered Medline: 21 Sep 2007

AB The tracking of cell fate, shape and migration is an essential component in the study of the development of multicellular organisms. Here we report a protocol that uses the protein Kaede, which is fluorescent green after synthesis but can be photoconverted red by violet or UV light. We have used Kaede along with confocal laser scanning microscopy to track labeled cells in a pattern of interest in zebrafish embryos. This technique allows the visualization of cell movements and the tracing of neuronal shapes. We provide illustrative examples of expression by mRNA injection, mosaic expression by DNA injection, and the creation of permanent transgenic fish with the UAS-Gal4 system to visualize morphogenetic processes such as neurulation, placode formation and navigation of early commissural axons in the hindbrain. The procedure can be adapted to other photoconvertible and reversible fluorescent molecules, including KikGR and Dronpa; these molecules can be used in combination with two-photon confocal microscopy to specifically highlight cells buried in tissues. The total time needed to carry out the protocol involving transient expression of Kaede by injection of mRNA or DNA, photoconversion and imaging is 2-8 d.

L4 ANSWER 5 OF 22 MEDLINE on STN
ACCESSION NUMBER: 2006452697 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16753144
TITLE: Dynamic behavior of individual cells in developing organotypic brain slices revealed by the photoconvertable protein Kaede.
AUTHOR: Mutoh T; Miyata T; Kashiwagi S; Miyawaki A; Ogawa M
CORPORATE SOURCE: Laboratory for Cell Culture Development, Advanced Technology Development Center, Brain Science Institute, Riken Saitama, Japan.. tmuto@brain.riken.jp
SOURCE: Experimental neurology, (2006 Aug) Vol. 200, No. 2, pp. 430-7. Electronic Publication: 2006-06-06.
Journal code: 0370712. ISSN: 0014-4886.
PUB. COUNTRY: United States
DOCUMENT TYPE: (COMPARATIVE STUDY)
(IN VITRO)
Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200609
ENTRY DATE: Entered STN: 1 Aug 2006
Last Updated on STN: 22 Sep 2006
Entered Medline: 21 Sep 2006

AB In recent years, advances in optical imaging methods have facilitated the visualization of events in the developing cortex. In particular, the introduction of DNA encoding fluorescent protein into cells of the embryonic brain allows the visualization of progenitor cells; slice preparations of the cortex then allow the monitoring of the behavior of transfected cells in the context of the living cerebral wall by time-lapse microscopy. Such approaches have provided substantial information about the patterns of neuronal migration. However, as these techniques label

large numbers of cells in the ventricular zone (VZ), it is difficult to follow individual cell shape changes or cell behaviors within the VZ, where neuron production and initial migration take place. Here, we report a unique method using the photoconvertable fluorescent protein Kaede, which emits green fluorescence and shifts to emitting red fluorescence upon radiation with UV. Using this method, we were able to follow the behavior of a particular pair of daughter cells among neighboring Kaede-positive cells in the SVZ of mouse brain slices. The spindle shape progenitor divided into two multipolar-shaped daughter cells. The cell-cell borders of daughter cells were clearly visualized, and easily describe the position and distance between two or more cells. The photoconvertable property of Kaede offers a powerful cell marking tool to identify the precise morphology and migratory behaviors of individual cells within living cortical slices.

L4 ANSWER 6 OF 22 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 2007:30318 BIOSIS
DOCUMENT NUMBER: PREV200700030032
TITLE: The 1.7 angstrom crystal structure of Dronpa: A photoswitchable green fluorescent protein.
AUTHOR(S): Wilmann, Pascal G.; Turcic, Kristina; Battad, Jion M.; Wilce, Matthew C. J.; Devenish, Rodney J.; Prescott, Mark [Reprint Author]; Rossjohn, Jamie
CORPORATE SOURCE: Monash Univ, Prot Crystallog Unit, Clayton, Vic 3800, Australia
Mark.Prescott@med.monash.edu.au; Jamie.Rossjohn@med.monash.edu.au
SOURCE: Journal of Molecular Biology, (NOV 24 2006) Vol. 364, No. 2, pp. 213-224.
CODEN: JMOBAK. ISSN: 0022-2836.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 27 Dec 2006
Last Updated on STN: 27 Dec 2006

AB The green fluorescent protein (GFP), its variants, and the closely related GFP-like proteins possess a wide variety of spectral properties that are of widespread interest as biological tools. One desirable spectral property termed photoswitching, involves the light-induced alteration of the optical properties of certain GFP members. Although the structural basis of both reversible and irreversible photoswitching events have begun to be unraveled, the mechanisms resulting in reversible photoswitching are less clear. A novel GFP-like protein, Dronpa, was identified to have remarkable light-induced photoswitching properties, maintaining an almost perfect reversible photochromic behavior with a high fluorescence to dark state ratio. We have crystallized and subsequently determined to 1.7 angstrom resolution the crystal structure of the fluorescent state of Dronpa. The chromophore was observed to be in its anionic form, adopting a cis co-planar conformation. Comparative structural analysis of non-photoactivatable and photoactivatable GFPs, together with site-directed mutagenesis of a position (Cys62) within the Dronpa chromophore, has provided a basis for understanding Dronpa photoactivation. Specifically, we propose a model of reversible photoactivation whereby irradiation with light leads to subtle conformational changes within and around the environment of the chromophore that promotes proton transfer along an intricate polar network. (c) 2006 Elsevier Ltd. All rights reserved.

L4 ANSWER 7 OF 22 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:569901 BIOSIS
DOCUMENT NUMBER: PREV200600582693
TITLE: Cloning and characterization of novel fluorescent proteins from Anthozoan animals and their applications to cell biological research.
AUTHOR(S): Karasawa, Satoshi; Miyawaki, Atsushi

SOURCE: Zoological Science (Tokyo), (DEC 2005) Vol. 22, No. 12, pp. 1417-1418.
Meeting Info.: 76th Annual Meeting of the Zoological-Society-of-Japan. Tsukuba, JAPAN. October 06 -08, 2005. Zool Soc Japan.
CODEN: ZOSCEX. ISSN: 0289-0003.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 1 Nov 2006
Last Updated on STN: 1 Nov 2006

L4 ANSWER 8 OF 22 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2005110192 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15731765
TITLE: Semi-rational engineering of a coral fluorescent protein into an efficient highlighter.
AUTHOR: Tsutsui Hidekazu; Karasawa Satoshi; Shimizu Hideaki; Nukina Nobuyuki; Miyawaki Atsushi
CORPORATE SOURCE: Laboratory for Cell Function Dynamics, Brain Science Institute, RIKEN, 2-1 Hirosawa, Wako-city, Saitama 351-0198, Japan.
SOURCE: EMBO reports, (2005 Mar) Vol. 6, No. 3, pp. 233-8.
Journal code: 100963049. ISSN: 1469-221X.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200508
ENTRY DATE: Entered STN: 3 Mar 2005
Last Updated on STN: 9 Aug 2005
Entered Medline: 8 Aug 2005

AB Kaede is a natural photoconvertible fluorescent protein found in the coral Trachyphyllia geoffroyi. It contains a tripeptide, His 62-Tyr 63-Gly 64, which acts as a green chromophore that is photoconvertible to red following (ultra-) violet irradiation. Here, we report the molecular cloning and crystal structure determination of a new fluorescent protein, KikG, from the coral Favia favus, and its in vitro evolution conferring green-to-red photoconvertibility. Substitution of the His 62-Tyr 63-Gly 64 sequence into the native protein provided only negligible photoconversion. On the basis of the crystal structure, semi-rational mutagenesis of the amino acids surrounding the chromophore was performed, leading to the generation of an efficient highlighter, KikGR. Within mammalian cells, KikGR is more efficiently photoconverted and is several-fold brighter in both the green and red states than Kaede. In addition, KikGR was successfully photoconverted using two-photon excitation microscopy at 760 nm, ensuring optical cell labelling with better spatial discrimination in thick and highly scattering tissues.

L4 ANSWER 9 OF 22 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 2005:483889 BIOSIS
DOCUMENT NUMBER: PREV200510287888
TITLE: Applications of fluorescent protein, kaede, to Arabidopsis.
AUTHOR(S): Yamamoto, Junko [Reprint Author]; Arimura, Shin-ichi; Tsutsumi, Nobuhiro
CORPORATE SOURCE: Univ Tokyo, Grad Sch Agr and Sci, Tokyo, Japan
SOURCE: Plant and Cell Physiology, (2005) Vol. 46, No. Suppl. S, pp. S148.
Meeting Info.: 46th Annual Meeting of the Japanese-Society-of-Plant-Physiologists. Niigata, JAPAN. March 24 -26, 2005. Japanese Soc Plant Physiologists.
CODEN: PCPHA5. ISSN: 0032-0781.
DOCUMENT TYPE: Conference; (Meeting)

Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 16 Nov 2005
Last Updated on STN: 16 Nov 2005

L4 ANSWER 10 OF 22 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
DUPLICATE 4
ACCESSION NUMBER: 2004-10115 BIOTECHDS
TITLE: Chromoprotein from Anthopleura inornata and fluoroproteins from Trachyphyllia geoffroyi and Scolymia vitiensis for optical marking of cells and organs; involving vector-mediated gene transfer and expression in host cell for use in cell and organ optical marking
AUTHOR: MIYAWAKI A; ANDO R; KARASAWA S; MIZUNO H
PATENT ASSIGNEE: RIKEN KK; MEDICAL and BIOLOGICAL LAB CO LTD
PATENT INFO: WO 2004018671 4 Mar 2004
APPLICATION INFO: WO 2003-JP10628 22 Aug 2003
PRIORITY INFO: JP 2002-280118 26 Sep 2002; JP 2002-243337 23 Aug 2002
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 2004-226849 [21]
AB DERWENT ABSTRACT:
NOVELTY - Chromoprotein derived from Anthopleura inornata and chromoproteins derived from it by addition, deletion and/or substitution of one or more amino acid residues, are new.

DETAILED DESCRIPTION - Chromoprotein derived from the coral Anthopleura inornata and chromoproteins derived from it by addition, deletion and/or substitution of one or more amino acid residues, are new. The chromoprotein has a working pH range of 5-10. The amino acid sequences of two forms of the chromoprotein are given having respectively an absorption maximum at 605nm (molar absorption 47550) and at 553nm (molar absorption 25300). INDEPENDENT CLAIMS are also given for: (1) fluoroprotein derived from the coral Trachyphyllia geoffroyi and fluoroproteins derived from it by addition, deletion and/or substitution of one or more amino acid residues. The fluoroprotein has absorption maxima at 508nm (molar absorption 98800) and 572nm (molar absorption 60400) and emission maxima at 518nm and 581nm with quantum yields of 0.80 and 0.33 respectively. The pKa is 5.7. (2) fluoroprotein derived from the coral Scolymia vitiensis and fluoroproteins derived from it by addition, deletion and/or substitution of one or more amino acid residues. The fluoroprotein has absorption maxima at 508nm (molar absorption 102250) and 578nm (molar absorption 76950) and emission maxima at 518nm and 588nm with quantum yields of 0.43 and 0.51 respectively. The pKa is 5.8 (508nm) or 6.5 (578nm); (3) DNA encoding the chromoprotein and fluoroproteins; (4) expression vectors containing this DNA; (5) hosts transformed by the vectors; (6) fusion proteins containing the chromoprotein or fluoroproteins together with another protein; (7) analysis of a biologically active protein and its function using the fluorescence resonance energy transition (FRET) method with the chromoprotein from Anthopleura inornata as acceptor; (8) analysis of function of a protein within the cell by expression of a fusion protein of the protein with the coral-derived chromoprotein or fluoroproteins within the cell; and (9) kits for these analysis methods.

USE - Optical marking of cells and organs and analysis of biologically active proteins and their function.

EXAMPLE - Total RNA is isolated from the coral Anthopleura inornata. The RNA is used to synthesize cDNA using a Ready-to-Go First Strand cDNA Synthesis Kit (Amersham-Pharmacia). The cDNA is amplified by polymerase chain reaction (PCR) using primer 5'-CCCGGATCCGACCATGGCTACCTTGGTTAAAGA-3' and oligo-dT primer, to yield a 1100bp fragment which is inserted into pRSET vector (Invitrogen) and used to transform Escherichia coli JM109-DE3. The transformant is cultured and the protein purified on Ni-Agarose gel (Qiagen) to give the chromoprotein (Be-G). The protein shows absorption maxima at 277nm and 605nm. (118 pages)

L4 ANSWER 11 OF 22 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
DUPLICATE 5
ACCESSION NUMBER: 2004-14562 BIOTECHDS
TITLE: New modified green fluorescent protein, useful as a reporter in expression studies;
modified fusion protein prepared by directed evolution useful as a reporter gene protein
AUTHOR: WALDO G S
PATENT ASSIGNEE: WALDO G S
PATENT INFO: US 2004078148 22 Apr 2004
APPLICATION INFO: US 2003-423688 24 Apr 2003
PRIORITY INFO: US 2003-423688 24 Apr 2003; US 2002-132067 24 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-340059 [31]
AB DERWENT ABSTRACT:
NOVELTY - A green fluorescent protein that comprises at least 80% identity to a fully defined sequence of 56 amino acids (SEQ ID NO:1); (b) at least one amino acid substitution selected from e.g., a substitution at position 30 that is an arginine or a conservative variant of arginine; and (c) measurable fluorescence activity.

DETAILED DESCRIPTION - A green fluorescent protein that comprises at least 80% identity to a fully defined sequence of 56 amino acids (SEQ ID NO:1); (b) at least one amino acid substitution selected from the group consisting of a substitution at position 30 that is an arginine or a conservative variant of arginine; a substitution at position 39 that is an asparagine or a conservative variant of asparagine; a substitution at position 105 that is a threonine or a conservative variant of threonine; a substitution at position 171 that is a valine; and a substitution at position 206 that is a valine; where the positions are determined in alignment for maximal correspondence with SEQ ID NO:1; and (c) measurable fluorescence activity. INDEPENDENT CLAIMS are also included for the following: (1) directed-evolution (M1) of generating enhanced folding variant of target polypeptide, comprising mutating a polynucleotide encoding polypeptide of interest to generate a library of mutated polynucleotides, linking mutated polynucleotides to polynucleotide encoding folding interference domain to form fusion protein (FP) constructs, expressing FP, and selecting FP that display optimal folding activity in relation to FP comprising wild-type polypeptide and folding interference domain, thus identifying polypeptide with enhanced folding activity. (2) an enhanced folding variant (I) of a fluorescent protein generated by (M1); (3) an enhanced folding variant (II) of a chromophoric protein generated by (M1); (4) enhancing (M2) folding of a polypeptide comprising multiple domains, involves joining a first domain of the polypeptide to a poorly folding domain, to form a fusion protein, mutating the first domain, detecting an increase in the amount of activity generated by a first mutated fusion protein in comparison to a fusion protein comprising a wild-type first domain and the poorly folding polypeptide domain, thus identifying a first domain with enhanced folding, joining a second domain of the polypeptide to the first mutated fusion protein to form a second fusion protein, mutating the second domain, and detecting an increase in the amount of activity generated by a second mutated fusion protein in comparison to a fusion protein comprising the wild-type second domain and the first mutated fusion protein, thus identifying a target polypeptide with multiple domains that have enhanced folding; (5) an isolated nucleic acid (IV) encoding a green fluorescent protein that has at least 80% identity to (S1), at least one amino acid substitution as described above, and measurable fluorescent activity; (6) an expression vector (V) comprising (IV); and (7) a host cell comprising (V).

BIOTECHNOLOGY - Preferred Methods: (M1) further involves carrying out one or more subsequent rounds of directed evolution on a fusion protein construct encoding an initially selected fusion protein that

display optimal folding activity, and selecting fusion proteins from it with optimal folding activity relative to the folding activity of the initially selected fusion protein, thus identifying polypeptides with further enhanced folding activity. The folding activity is determined by measuring folding kinetics or by measuring resistance to denaturation by urea. The folding activity of the fusion protein is determined by measuring a biological activity of the target polypeptide. The target polypeptide is a fluorescent protein and the biological activity is fluorescence. The target polypeptide is a chromophoric protein and the biological activity is color. The fluorescent protein is chosen from Aequorea victoria green fluorescent protein and a fluorescent protein having a structure with a root mean square deviation of less than 5Angstrom from the 11- stranded beta-barrel component of the A.victoria Green fluorescent protein structure MMDB Id: 5742. Preferred Variant: In (I), the fluorescent protein is A.victoria green fluorescent protein, red fluorescent protein from a Discosoma species, Anthosoan fluorescent protein, Trachyphyllia geoffroyi, or Anemonia sulcata. In (II), the fluorescent protein has a fully defined amino acid sequence of 182 amino acids (S2), or 168 amino acids (S3). The target polypeptide is a reporter polypeptide. The fusion protein further comprises a reporter molecule that has the reporter activity. The reporter activity is chosen from fluorescent signal or antibiotic resistance. The poorly folding domain is a ferritin domain. The fusion protein comprises a target polypeptide linked to the poorly folding domain by a linker. The reporter molecule is linked to the fusion protein. The folding interference domain is inserted into permissive sites of the target polypeptide. The target polypeptide is green fluorescent protein that has at least 80% identity when aligned for maximum correspondence to (S2) or to a fully defined sequence of 180 amino acids (S4), and has fluorescent activity. Preferred Protein: (III) further comprises a phenylalanine substitution at position 145. The amino acid substitution is chosen from arginine substitution at position 30, an asparagine substitution position 39, threonine substitution at position 105, valine substitution at position 171, and a valine substitution at position 206. The substitution is arginine at position 30, asparagine at position 39, threonine at position 105, phenylalanine at position 145, valine at position 171, or valine at position 206. The green fluorescent protein comprises any two, three, four, or five substitutions as described in (III). The five substitutions are an arginine at position 30, asparagine at position 39, threonine at position 105, valine at position 171 and valine at position 206. The green fluorescent protein further comprises a sixth substitution that is a phenylalanine at position 145. (III) further comprises a mutation chosen from Phe99Ser, Met153Thr and Val163Ala. (III) is cyclized. Preferred Nucleic Acid: (IV) encodes a green fluorescent protein having at least one amino acid substitution chosen from arginine substitution at position 30, asparagine substitution at position 39, threonine substitution at position 105, phenylalanine substitution at position 145, valine substitution at position 171, and a valine substitution at position 206.

USE - (I) and (II) are useful as reporter proteins to express the report level of a protein. (M1) is useful for directed-evolution of generating enhanced folding variant of target polypeptide (claimed). (M1) is useful for improving folding and solubility of a target protein.

ADVANTAGE - (I) provides new and more stable scaffolds for the creation of new GFP variants.

EXAMPLE - To test the effect of the superfolder mutations, 6 single-point mutants of cycle-3 redshift were engineered by PCR. Each mutant incorporated one of the 6 mutations found the superholder green fluorescent protein (GFP) variant. These were cloned into a pET vector as C-terminal fusions with poorly-folded bullfrog redcell ferritin. Rapid protein-folding assay using green fluorescent protein was carried out. Overnight cultures in Luria-Bertani (LB) media containing kanamycin (35 g/ml) were diluted 100-fold and grown for 2 hours at 37degreesC. Proteins were expressed for 4 hours by adding isopropyl-D-thiogalactopyranoside

(IPTG) to 1 mM in 3 ml cultures of LB media at either 37degreesC or 27degreesC in Escherichia coli BL21(DE3) as C-terminal fusions with poorly-folded bull frog red cell H-subunit ferritin. Cycle-3 redshift and superfolder were cloned and expressed similarly as controls, both with and without the N-terminal ferritin. The fluorescence (488 nm ex/520 nm em) and absorbance (600 nm) were measured for each culture using a BioTek FL-600 plate reader. (46 pages)

L4 ANSWER 12 OF 22 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2004:154122 HCAPLUS
DOCUMENT NUMBER: 140:318335
TITLE: Conventional taxonomy obscures deep divergence between Pacific and Atlantic corals OPEN-TID: : 0
AUTHOR(S): Fukami, Hironobu; Budd, Ann F.; Paulay, Gustav; Sole-Cava, Antonio; Chen, Chaolun Allen; Iwao, Kenji; Knowlton, Nancy
CORPORATE SOURCE: Smithsonian Tropical Research Institute, Naos Marine Laboratory, Balboa, Panama
SOURCE: Nature (London, United Kingdom) (2004), 427(6977), 832-835
CODEN: NATUAS; ISSN: 0028-0836
PUBLISHER: Nature Publishing Group
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Only 17% of 111 reef-building coral genera and none of the 18 coral families with reef-builders are considered endemic to the Atlantic, whereas the corresponding percentages for the Indo-west Pacific are 76% and 39%. These figures depend on the assumption that genera and families spanning the 2 provinces belong to the same lineages (i.e., they are monophyletic). Here we show that this assumption is incorrect on the basis of analyses of mitochondrial and nuclear genes. Pervasive morphol. convergence at the family level has obscured the evolutionary distinctiveness of Atlantic corals. Some Atlantic genera conventionally assigned to different families are more closely related to each other than they are to their resp. Pacific congeners'. Nine of the 27 genera of reef-building Atlantic corals belong to this previously unrecognized lineage, which probably diverged >34 million yr ago. Although Pacific reefs have larger nos. of more narrowly distributed species, and therefore rank higher in biodiversity hotspot analyses, the deep evolutionary distinctiveness of many Atlantic corals should also be considered when setting conservation priorities.
REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 13 OF 22 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2003:886090 HCAPLUS
DOCUMENT NUMBER: 140:107188
TITLE: Photo-induced peptide cleavage in the green-to-red conversion of a fluorescent protein
AUTHOR(S): Mizuno, Hideaki; Mal, Tapas Kumar; Tong, Kit I.; Ando, Ryoko; Furuta, Toshiaki; Ikura, Mitsuhiko; Miyawaki, Atsushi
CORPORATE SOURCE: Laboratory for Cell Function Dynamics, Advanced Technology Development Group, Brain Science Institute, The Institute of Physical and Chemical Science (RIKEN), Wako-city, 351-0198, Japan
SOURCE: Molecular Cell (2003), 12(4), 1051-1058
CODEN: MOCEFL; ISSN: 1097-2765
PUBLISHER: Cell Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Green fluorescent protein from the jellyfish (Aequorea GFP) and GFP-like proteins from coral species encode light-absorbing chromophores within their protein sequences. A coral fluorescent protein, Kaede, contains a

tripeptide, His62-Tyr63-Gly64, which acts as a green chromophore that is photoconverted to red. Here, the authors present the structural basis for the green-to-red photoconversion. As in Aequorea GFP, a chromophore, 4-(p-hydroxybenzylidene)-5-imidazolinone, derived from the tripeptide mediates green fluorescence in Kaede. UV irradiation causes an unconventional cleavage within Kaede protein between the amide nitrogen and the α carbon (Cα) at His62 via a formal β-elimination reaction, which requires the whole, intact protein for its catalysis. The subsequent formation of a double bond between His62-Cα and -Cβ extends the π-conjugation to the imidazole ring of His62, creating a new red-emitting chromophore, 2-[(1E)-2-(5-imidazolyl)ethenyl]-4-(p-hydroxybenzylidene)-5-imidazolinone. The present study not only reveals diversity in the chemical structure of fluorescent proteins but also adds a new dimension to posttranslational modification mechanisms.

REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 14 OF 22 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2003:342964 HCAPLUS
DOCUMENT NUMBER: 139:256466
TITLE: Changes in zooxanthellae density, morphology, and mitotic index in hermatypic corals and anemones exposed to cyanide
AUTHOR(S): Cervino, J. M.; Hayes, R. L.; Honovich, M.; Goreau, T. J.; Jones, S.; Rubec, P. J.
CORPORATE SOURCE: University of South Carolina, Columbia, SC, 29801, USA
SOURCE: Marine Pollution Bulletin (2003), 46(5), 573-586
CODEN: MPNBAZ; ISSN: 0025-326X
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Sodium cyanide (NaCN) is widely used for the capture of reef fish throughout Southeast Asia and causes extensive fish mortality, but the effect of NaCN on reef corals remains debated. To document the impact of cyanide exposure on corals, the species Acropora millepora, Goniopora sp., Favites abdita, Trachyphyllia geoffrio, Plerogyra sp., Heliofungia actinformis, Euphyllia divisa, and Scarophyton sp., and the sea anemone Aiptasia pallida were exposed to varying concns. of cyanide for varying time periods. Corals were exposed to 50, 100, 300, and 600 mg/l of cyanide ion (CN-) for 1-2 min (in seawater, the CN- forms hydrocyanic acid). These concns. are much lower than those reportedly used by fish collectors. Exposed corals and anemones immediately retracted their tentacles and mesenterial filaments, and discharged copious amts. of mucus containing zooxanthellae. Gel electrophoreses techniques found changes in protein expression in both zooxanthellae and host tissue. Corals and anemones exposed to cyanide showed an immediate increase in mitotic cell division of their zooxenthellae, and a decrease in zooxanthellae d. In contrast, zooxanthellae cell division and d. remained constant in controls. Histopathol. changes included gastrodermal disruption, mesogleal degradation, and increased mucus in coral tissues. Zooxanthellae showed pigment loss, swelling, and deformation. Mortality occurred at all exposure levels. Exposed specimens experienced an increase in the ratio of gram-neg. to gram-pos. bacteria on the coral surface. The results demonstrate that exposure cyanide causes mortality to corals and anemones, even when applied at lower levels than that used by fish collectors. Even brief exposure to cyanide caused slow-acting and long-term damage to corals and their zooxanthellae.

REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 15 OF 22 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 2002616608 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12271129
TITLE: An optical marker based on the UV-induced green-to-red

photoconversion of a fluorescent protein.
AUTHOR: Ando Ryoko; Hama Hiroshi; Yamamoto-Hino Miki; Mizuno Hideaki; Miyawaki Atsushi
CORPORATE SOURCE: Laboratory for Cell Function and Dynamics, Advanced Technology Development Center, Brain Science Institute, The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako-city, Saitama 351-0198, Japan.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (2002 Oct 1) Vol. 99, No. 20, pp. 12651-6. Electronic Publication: 2002-09-23.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AB085641
ENTRY MONTH: 200211
ENTRY DATE: Entered STN: 11 Oct 2002
Last Updated on STN: 5 Jan 2003
Entered Medline: 13 Nov 2002

AB We have cloned a gene encoding a fluorescent protein from a stony coral, Trachyphyllia geoffroyi, which emits green, yellow, and red light. The protein, named Kaede, includes a tripeptide, His-Tyr-Gly, that acts as a green chromophore that can be converted to red. The red fluorescence is comparable in intensity to the green and is stable under usual aerobic conditions. We found that the green-red conversion is highly sensitive to irradiation with UV or violet light (350-400 nm), which excites the protonated form of the chromophore. The excitation lights used to elicit red and green fluorescence do not induce photoconversion. Under a conventional epifluorescence microscope, Kaede protein expressed in HeLa cells turned red in a graded fashion in response to UV illumination; maximal illumination resulted in a 2,000-fold increase in the ratio of red-to-green signal. These color-changing properties provide a simple and powerful technique for regional optical marking. A focused UV pulse creates an instantaneous plane source of red Kaede within the cytosol. The red spot spreads rapidly throughout the cytosol, indicating its free diffusibility in the compartment. The extensive diffusion allows us to delineate a single neuron in a dense culture, where processes originating from many different somata are present. Illumination of a focused UV pulse onto the soma of a Kaede-expressing neuron resulted in filling of all processes with red fluorescence, allowing visualization of contact sites between the red and green neurons of interest.

L4 ANSWER 16 OF 22 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1988:29215 BIOSIS
DOCUMENT NUMBER: PREV198885016940; BA85:16940
TITLE: NEW OBSERVATIONS ON SCLERACTINIAN CORALS FROM INDONESIA 1. FREE-LIVING SPECIES BELONGING TO THE FAVIINA.
AUTHOR(S): BEST M B [Reprint author]; HOEKSEMA B W
CORPORATE SOURCE: RIJKSMUSEUM NATUURLIJKE HISTORIE, POSTBUS 9517, 2300 RA LEIDEN, NETHERLANDS
SOURCE: Zoologische Mededelingen (Leiden), (1987) Vol. 61, No. 27, pp. 387-403.
ISSN: 0024-0672.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 28 Dec 1987
Last Updated on STN: 28 Dec 1987

AB Five free-living coral species (one new) belonging to four genera (one thus far only known fossil), and their adaptation to soft substrata are

discussed. The species discussed are [Trachyphyllia geoffroyi, T. radiata, Indophyllia macassarensis sp. nov, Cynarina lacrymalis, and Anthemiphyllia dentata].

L4 ANSWER 17 OF 22 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 1984:168767 BIOSIS
DOCUMENT NUMBER: PREV198477001751; BA77:1751
TITLE: FREE LIVING CORALS DISTRIBUTIONS ACCORDING TO PLANT COVER SEDIMENTS HYDRODYNAMICS DEPTH AND BIOLOGICAL FACTORS.
AUTHOR(S): FISK D A [Reprint author]
CORPORATE SOURCE: AUSTRALIAN INST MARINE SCI, PMB 3, TOWNSVILLE MSO, TOWNSVILLE, QUEENSLAND 4810, AUSTRALIA
SOURCE: Marine Biology (Berlin), (1983) Vol. 74, No. 3, pp. 287-294.
CODEN: MBIOAJ. ISSN: 0025-3162.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH

AB In 1974, 8 free-living coral spp. were found to inhabit the sandy sea floor adjacent to Lizard Island, Great Barrier Reef (14°40'S; 145°78'E). They fall into 2 groups which colonize 2 dissimilar sediment types. Plant cover increases with depth and, because of its effect on sediment characteristics, is thought to be a significant factor affecting coral distributions. The shallower coarse to medium grain sediments (0.5-0.125 mm) are mainly colonized by Heteropsammia cochlea, Heterocyathus aequicostatus, Diaseris distorta, and to a lesser extent by Cycloseris cyclolites. The deeper sediments are made up of a biogenically derived coarse fraction (larger than 0.5 mm) combined with an equally high proportion of fine-grade material. Corals typically found on these sediments are: Trachyphyllia geoffroyi, Catalaphyllia jardinei, Cynarina lacrymalis and Cycloseris patelliformis. The content of non-carbonate material in the sediments reflects the hydrodynamics of the area and hence the degree of sedimentation, i.e., traction, saltation or suspension loads, the corals have to cope with. Depth of occurrence predicted local coral distributions but was not applicable to other regions. Other factors which are discussed in relation to coral distributions include: coral mobility, coral shape, the effect of an obligate sipunculan associate, Aspidosiphon jukesii, in H. cochlea and H. aequicostatus and settlement requirements.

L4 ANSWER 18 OF 22 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 1980:176344 BIOSIS
DOCUMENT NUMBER: PREV198069051340; BA69:51340
TITLE: MUCUS ANTIGENICITY IN SEA-ANEMONES AND CORALS.
AUTHOR(S): LUBBOCK R [Reprint author]
CORPORATE SOURCE: DEP ZOOL, UNIV CAMB, DOWNING ST, CAMBRIDGE CB2 3EJ, ENGL, UK
SOURCE: Hydrobiologia, (1979) Vol. 66, No. 1, pp. 3-6.
CODEN: HYDRB8. ISSN: 0018-8158.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH

AB The antigenicities of external mucus from the sea anemones Stoichactis haddoni, Radianthus ritteri and Gyrostoma hertwigi and from the coral Trachyphyllia geoffroyi were compared. Marked differences were found between species but not within species. Mucus composition might be species specific and may be one of the factors used by sea anemones and corals in the recognition of foreign anthozoan species.

L4 ANSWER 19 OF 22 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 1981:26954 BIOSIS
DOCUMENT NUMBER: PREV198120026954; BR20:26954
TITLE: SCLERACTINIA OF EASTERN AUSTRALIA 2. FAMILIES FAVIIDAE TRACHYPHYLLIIDAE.
AUTHOR(S): VERON J E N [Reprint author]; PICHON M; WIJSMAN-BEST M
CORPORATE SOURCE: AUST INST MARINE SCI, TOWNSVILLE, AUST
SOURCE: Australian Institute of Marine Science Monograph Series, (1977) Vol. 3, pp. 1-233.
ISSN: 1037-3047.
DOCUMENT TYPE: Article
FILE SEGMENT: BR
LANGUAGE: ENGLISH

L4 ANSWER 20 OF 22 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1975:60245 BIOSIS
DOCUMENT NUMBER: PREV197511060245; BR11:60245
TITLE: FREE LIVING SCLERACTINIAN CORAL COMMUNITIES IN THE CORAL REEFS OF MADAGASCAR MALAGASY-REPUBLIC.
AUTHOR(S): PICHON M
SOURCE: (1974) pp. 173-181. CAMERON, A. M. ET AL (ED.). PROCEEDINGS OF THE SECOND INTERNATIONAL SYMPOSIUM ON CORAL REEFS, VOL 2. JUNE 22-JULY 2, 1973. 753P. ILLUS. MAPS. THE GREAT BARRIER REEF COMMITTEE: BRISBANE, AUSTRALIA. ISBN 0-909377-02-4.
DOCUMENT TYPE: Book
FILE SEGMENT: BR
LANGUAGE: Unavailable

L4 ANSWER 21 OF 22 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1952:10695 BIOSIS
DOCUMENT NUMBER: PREV19522600010721; BA26:10721
TITLE: A revision of the Teleosauridae in the Oxford University Museum and the British Museum (Natural History).
AUTHOR(S): PHIZACKERLEY, P. H.
CORPORATE SOURCE: U. Oxford
SOURCE: ANN AND MAG NAT HIST 12TH SER, (1951) Vol. 4, No. 48, pp. 1169-1192.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: Unavailable
ENTRY DATE: Entered STN: May 2007
Last Updated on STN: May 2007
AB An attempt to clarify the nomenclature of the Jurassic crocodiles represented by the genera Teleosaurus and Steneosaurus. The specimens in the Oxford Mus. are now of increased valuè as much of the type material, formerly in the Mus. at Caen, was destroyed in the last war. The taxonomic history is reviewed. The generic diagnoses are supplemented with detailed descrs. of T. cadomensis. T. geoffroyi. S. megistorhynchus. S. boutilieri, S. meretrix from the Great Oolite of Enslow Bridge, England, and S. depressus from the Oxford Clay of Peterborough, England.

L4 ANSWER 22 OF 22 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1995:486145 BIOSIS
DOCUMENT NUMBER: PREV199598500445
TITLE: New records of molluscs (Leptoconchus, Lithophaga, Fungiacava) that bore Indo-Pacific reef corals and their interactions with their hosts.
AUTHOR(S): Zibrowius, Helmut [Reprint author]; Arnaud, Patrick M.
CORPORATE SOURCE: Centre d'Oceanol. Marseille, Station Marine d'Endoume, rue Batterie-des-Lions, F-13007 Marseille, France

SOURCE: Bulletin du Museum National d'Histoire Naturelle Section A Zoologie Biologie et Ecologie Animales, (1994 (1995)) Vol. 16, No. 2-4, pp. 231-244. .
ISSN: 0181-0626.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 9 Nov 1995
Last Updated on STN: 9 Nov 1995

AB New records of the coralliophilid gastropod Leptoconchus striatus in Fungia somervillei from the Seychelles and of the mytilid bivalve Lithophaga lima in Trachyphyllia geoffroyi from the Philippines fall within the previously known geographical range. New records of the mytilid bivalve Fungiacava eilatensis in Fungia fragilis, from Reunion Island and Madagascar, considerably extend the range of this highly cryptic and therefore rarely recorded borer. The fungiid host's conformation appears to influence the shape of the Fungiacava individuals. Fungiacava may be inaequivalve. Calcareous deposits lining the cavity of the coralliophilid are produced by the coral host, whereas those lining the cavities of both mytilids are produced by the borers. Growing to an excessive size with respect to its coral host in this particular case, L. lima had to shift position, its migrating cavity aperture leaving behind a sealed track. This suggests a comparison with the boring behaviour observed in an acrothoracid cirriped (Lithoglyptes viatrix) infesting deep-water corals.

=> d his

(FILE 'HOME' ENTERED AT 10:20:28 ON 05 OCT 2007)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:21:01 ON 05 OCT 2007

L1 1378 S FLUOROSCEN?
L2 38 S TRACHYPHYLLIA (W) GEOFFROYI
L3 0 S L1 AND L2
L4 22 DUP REM L2 (16 DUPLICATES REMOVED)

=> s l4 and green
L5 12 L4 AND GREEN

=> d 1-12 ibib ab

L5 ANSWER 1 OF 12 MEDLINE on STN
ACCESSION NUMBER: 2007530894 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 17692334
TITLE: Crystallographic Evidence for Water-assisted Photo-induced Peptide Cleavage in the Stony Coral Fluorescent Protein Kaede.
AUTHOR: Hayashi Ikuko; Mizuno Hideaki; Tong Kit I; Furuta Toshiaki; Tanaka Fujie; Yoshimura Masato; Miyawaki Atsushi; Ikura Mitsuhiko
CORPORATE SOURCE: Division of Signaling Biology, Ontario Cancer Institute and Department of Medical Biophysics, 101 College St., Toronto, Ontario, M5G 1L7, Canada.
SOURCE: Journal of molecular biology, (2007 Sep 28) Vol. 372, No. 4, pp. 918-26. Electronic Publication: 2007-06-19.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: NONMEDLINE; IN-DATA-REVIEW; IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 12 Sep 2007
Last Updated on STN: 12 Sep 2007

AB A coral fluorescent protein from Trachyphyllia geoffroyi , Kaede, possesses a tripeptide of His62-Tyr63-Gly64, which forms a chromophore with green fluorescence. This chromophore's fluorescence turns red following UV light irradiation. We have previously shown that such photoconversion is achieved by a formal beta-elimination reaction, which results in a cleavage of the peptide bond found between the amide nitrogen and the alpha-carbon at His62. However, the stereochemical arrangement of the chromophore and the precise structural basis for this reaction mechanism previously remained unknown. Here, we report the crystal structures of the green and red form of Kaede at 1.4 A and 1.6 A resolutions, respectively. Our structures depict the cleaved peptide bond in the red form. The chromophore conformations both in the green and red forms are similar, except a well-defined water molecule in the proximity of the His62 imidazole ring in the green form. We propose a molecular mechanism for green -to-red photoconversion, which is assisted by the water molecule.

L5 ANSWER 2 OF 12 MEDLINE on STN
ACCESSION NUMBER: 2007204166 MEDLINE
DOCUMENT NUMBER: PubMed ID: 17406330
TITLE: Cell tracking using a photoconvertible fluorescent protein.
AUTHOR: Hatta Kohei; Tsujii Hitomi; Omura Tomomi
CORPORATE SOURCE: Laboratory for Vertebrate Body Plan, Center for Developmental Biology, RIKEN Kobe 2-2-3 Minatojima Minamimachi, Chuo-ku, Kobe 650-0047, Japan.. khatta@cdb.riken.jp
SOURCE: Nature protocols, (2006) Vol. 1, No. 2, pp. 960-7. Journal code: 101284307. E-ISSN: 1750-2799.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200709
ENTRY DATE: Entered STN: 5 Apr 2007
Last Updated on STN: 22 Sep 2007
Entered Medline: 21 Sep 2007

AB The tracking of cell fate, shape and migration is an essential component in the study of the development of multicellular organisms. Here we report a protocol that uses the protein Kaede, which is fluorescent green after synthesis but can be photoconverted red by violet or UV light. We have used Kaede along with confocal laser scanning microscopy to track labeled cells in a pattern of interest in zebrafish embryos. This technique allows the visualization of cell movements and the tracing of neuronal shapes. We provide illustrative examples of expression by mRNA injection, mosaic expression by DNA injection, and the creation of permanent transgenic fish with the UAS-Gal4 system to visualize morphogenetic processes such as neurulation, placode formation and navigation of early commissural axons in the hindbrain. The procedure can be adapted to other photoconvertible and reversible fluorescent molecules, including KikGR and Dronpa; these molecules can be used in combination with two-photon confocal microscopy to specifically highlight cells buried in tissues. The total time needed to carry out the protocol involving transient expression of Kaede by injection of mRNA or DNA, photoconversion and imaging is 2-8 d.

L5 ANSWER 3 OF 12 MEDLINE on STN
ACCESSION NUMBER: 2006712529 MEDLINE
DOCUMENT NUMBER: PubMed ID: 17092037
TITLE: Competition between energy and proton transfer in ultrafast excited-state dynamics of an oligomeric fluorescent protein red Kaede.
AUTHOR: Hosoi Haruko; Mizuno Hideaki; Miyawaki Atsushi; Tahara Tahei

CORPORATE SOURCE: Molecular Spectroscopy Laboratory, RIKEN (The Institute of Physical and Chemical Research), Wako, Saitama 351-0198, Japan.
SOURCE: The journal of physical chemistry. B, (2006 Nov 16) Vol. 110, No. 45, pp. 22853-60.
Journal code: 101157530. ISSN: 1520-6106.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200701
ENTRY DATE: Entered STN: 12 Dec 2006
Last Updated on STN: 27 Jan 2007
Entered Medline: 26 Jan 2007

AB We investigated femtosecond and picosecond time-resolved fluorescence dynamics of a tetrameric fluorescent protein Kaede with a red chromophore (red Kaede) to examine a relationship between the excited-state dynamics and a quaternary structure of the fluorescent protein. Red Kaede was obtained by photoconversion from green Kaede that was cloned from a stony coral Trachyphyllia geoffroyi. In common with other typical fluorescent proteins, a chromophore of red Kaede has two protonation states, the neutral and the anionic forms in equilibrium. Time-resolved fluorescence measurements clarified that excitation of the neutral form gives the anionic excited state with a time constant of 13 ps at pH 7.5. This conversion process was attributed to fluorescence resonance energy transfer (FRET) from the photoexcited neutral form to the ground-state anionic form that is located in an adjacent subunit in the tetramer. The time-resolved fluorescence data measured at different pH revealed that excited-state proton transfer (ESPT) also occurs with a time constant of 300 ps and hence that the FRET and ESPT take place simultaneously in the fluorescent protein as competing processes. The ESPT rate in red Kaede was significantly slower than the rate in Aequorea GFP, which highly likely arises from the different hydrogen bond network around the chromophore.

L5 ANSWER 4 OF 12 MEDLINE on STN
ACCESSION NUMBER: 2006452697 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16753144
TITLE: Dynamic behavior of individual cells in developing organotypic brain slices revealed by the photoconvertable protein Kaede.
AUTHOR: Mutoh T; Miyata T; Kashiwagi S; Miyawaki A; Ogawa M
CORPORATE SOURCE: Laboratory for Cell Culture Development, Advanced Technology Development Center, Brain Science Institute, Riken Saitama, Japan.. tmuto@brain.riken.jp
SOURCE: Experimental neurology, (2006 Aug) Vol. 200, No. 2, pp. 430-7. Electronic Publication: 2006-06-06.
Journal code: 0370712. ISSN: 0014-4886.
PUB. COUNTRY: United States
DOCUMENT TYPE: (COMPARATIVE STUDY)
(IN VITRO)
Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200609
ENTRY DATE: Entered STN: 1 Aug 2006
Last Updated on STN: 22 Sep 2006
Entered Medline: 21 Sep 2006

AB In recent years, advances in optical imaging methods have facilitated the visualization of events in the developing cortex. In particular, the introduction of DNA encoding fluorescent protein into cells of the embryonic brain allows the visualization of progenitor cells; slice

preparations of the cortex then allow the monitoring of the behavior of transfected cells in the context of the living cerebral wall by time-lapse microscopy. Such approaches have provided substantial information about the patterns of neuronal migration. However, as these techniques label large numbers of cells in the ventricular zone (VZ), it is difficult to follow individual cell shape changes or cell behaviors within the VZ, where neuron production and initial migration take place. Here, we report a unique method using the photoconvertable fluorescent protein Kaede, which emits green fluorescence and shifts to emitting red fluorescence upon radiation with UV. Using this method, we were able to follow the behavior of a particular pair of daughter cells among neighboring Kaede-positive cells in the SVZ of mouse brain slices. The spindle shape progenitor divided into two multipolar-shaped daughter cells. The cell-cell borders of daughter cells were clearly visualized, and easily describe the position and distance between two or more cells. The photoconvertable property of Kaede offers a powerful cell marking tool to identify the precise morphology and migratory behaviors of individual cells within living cortical slices.

L5 ANSWER 5 OF 12 MEDLINE on STN
ACCESSION NUMBER: 2005110192 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15731765
TITLE: Semi-rational engineering of a coral fluorescent protein into an efficient highlighter.
AUTHOR: Tsutsui Hidekazu; Karasawa Satoshi; Shimizu Hideaki; Nukina Nobuyuki; Miyawaki Atsushi
CORPORATE SOURCE: Laboratory for Cell Function Dynamics, Brain Science Institute, RIKEN, 2-1 Hirosawa, Wako-city, Saitama 351-0198, Japan.
SOURCE: EMBO reports, (2005 Mar) Vol. 6, No. 3, pp. 233-8. Journal code: 100963049. ISSN: 1469-221X.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200508
ENTRY DATE: Entered STN: 3 Mar 2005
Last Updated on STN: 9 Aug 2005
Entered Medline: 8 Aug 2005

AB Kaede is a natural photoconvertible fluorescent protein found in the coral Trachyphyllia geoffroyi. It contains a tripeptide, His 62-Tyr 63-Gly 64, which acts as a green chromophore that is photoconvertible to red following (ultra-) violet irradiation. Here, we report the molecular cloning and crystal structure determination of a new fluorescent protein, KikG, from the coral Favia favus, and its in vitro evolution conferring green-to-red photoconvertibility. Substitution of the His 62-Tyr 63-Gly 64 sequence into the native protein provided only negligible photoconversion. On the basis of the crystal structure, semi-rational mutagenesis of the amino acids surrounding the chromophore was performed, leading to the generation of an efficient highlighter, KikGR. Within mammalian cells, KikGR is more efficiently photoconverted and is several-fold brighter in both the green and red states than Kaede. In addition, KikGR was successfully photoconverted using two-photon excitation microscopy at 760 nm, ensuring optical cell labelling with better spatial discrimination in thick and highly scattering tissues.

L5 ANSWER 6 OF 12 MEDLINE on STN
ACCESSION NUMBER: 2002616608 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12271129
TITLE: An optical marker based on the UV-induced green -to-red photoconversion of a fluorescent protein.
AUTHOR: Ando Ryoko; Hama Hiroshi; Yamamoto-Hino Miki; Mizuno

Hideaki; Miyawaki Atsushi
CORPORATE SOURCE: Laboratory for Cell Function and Dynamics, Advanced Technology Development Center, Brain Science Institute, The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako-city, Saitama 351-0198, Japan.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (2002 Oct 1) Vol. 99, No. 20, pp. 12651-6. Electronic Publication: 2002-09-23.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AB085641
ENTRY MONTH: 200211
ENTRY DATE: Entered STN: 11 Oct 2002
Last Updated on STN: 5 Jan 2003
Entered Medline: 13 Nov 2002

AB We have cloned a gene encoding a fluorescent protein from a stony coral, Trachyphyllia geoffroyi, which emits green, yellow, and red light. The protein, named Kaede, includes a tripeptide, His-Tyr-Gly, that acts as a green chromophore that can be converted to red. The red fluorescence is comparable in intensity to the green and is stable under usual aerobic conditions. We found that the green-red conversion is highly sensitive to irradiation with UV or violet light (350-400 nm), which excites the protonated form of the chromophore. The excitation lights used to elicit red and green fluorescence do not induce photoconversion. Under a conventional epifluorescence microscope, Kaede protein expressed in HeLa cells turned red in a graded fashion in response to UV illumination; maximal illumination resulted in a 2,000-fold increase in the ratio of red-to-green signal. These color-changing properties provide a simple and powerful technique for regional optical marking. A focused UV pulse creates an instantaneous plane source of red Kaede within the cytosol. The red spot spreads rapidly throughout the cytosol, indicating its free diffusibility in the compartment. The extensive diffusion allows us to delineate a single neuron in a dense culture, where processes originating from many different somata are present. Illumination of a focused UV pulse onto the soma of a Kaede-expressing neuron resulted in filling of all processes with red fluorescence, allowing visualization of contact sites between the red and green neurons of interest.

L5 ANSWER 7 OF 12 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 2007:30318 BIOSIS
DOCUMENT NUMBER: PREV200700030032
TITLE: The 1.7 angstrom crystal structure of Dronpa: A photoswitchable green fluorescent protein.
AUTHOR(S): Wilmann, Pascal G.; Turcic, Kristina; Battad, Jion M.; Wilce, Matthew C. J.; Devenish, Rodney J.; Prescott, Mark [Reprint Author]; Rossjohn, Jamie
CORPORATE SOURCE: Monash Univ, Prot Crystallog Unit, Clayton, Vic 3800, Australia
Mark.Prescott@med.monash.edu.au; Jamie.Rossjohn@med.monash.edu.au
SOURCE: Journal of Molecular Biology, (NOV 24 2006) Vol. 364, No. 2, pp. 213-224.
CODEN: JMOBAK. ISSN: 0022-2836.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 27 Dec 2006
Last Updated on STN: 27 Dec 2006

AB The green fluorescent protein (GFP), its variants, and the closely related GFP-like proteins possess a wide variety of spectral

properties that are of widespread interest as biological tools. One desirable spectral property termed photoswitching, involves the light-induced alteration of the optical properties of certain GFP members. Although the structural basis of both reversible and irreversible photoswitching events have begun to be unraveled, the mechanisms resulting in reversible photoswitching are less clear. A novel GFP-like protein, Dronpa, was identified to have remarkable light-induced photoswitching properties, maintaining an almost perfect reversible photochromic behavior with a high fluorescence to dark state ratio. We have crystallized and subsequently determined to 1.7 angstrom resolution the crystal structure of the fluorescent state of Dronpa. The chromophore was observed to be in its anionic form, adopting a cis co-planar conformation. Comparative structural analysis of non-photoactivatable and photoactivatable GFPs, together with site-directed mutagenesis of a position (Cys62) within the Dronpa chromophore, has provided a basis for understanding Dronpa photoactivation. Specifically, we propose a model of reversible photoactivation whereby irradiation with light leads to subtle conformational changes within and around the environment of the chromophore that promotes proton transfer along an intricate polar network.

L5 ANSWER 8 OF 12 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:569901 BIOSIS
DOCUMENT NUMBER: PREV200600582693
TITLE: Cloning and characterization of novel fluorescent proteins from Anthozoan animals and their applications to cell biological research.
AUTHOR(S): Karasawa, Satoshi; Miyawaki, Atsushi
SOURCE: Zoological Science (Tokyo), (DEC 2005) Vol. 22, No. 12, pp. 1417-1418.
Meeting Info.: 76th Annual Meeting of the Zoological-Society-of-Japan. Tsukuba, JAPAN. October 06 -08, 2005. Zool Soc Japan.
CODEN: ZOSCEX. ISSN: 0289-0003.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 1 Nov 2006
Last Updated on STN: 1 Nov 2006

L5 ANSWER 9 OF 12 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 2005:483889 BIOSIS
DOCUMENT NUMBER: PREV200510287888
TITLE: Applications of fluorescent protein, kaede, to Arabidopsis.
AUTHOR(S): Yamamoto, Junko [Reprint Author]; Arimura, Shin-ichi; Tsutsumi, Nobuhiro
CORPORATE SOURCE: Univ Tokyo, Grad Sch Agr and Sci, Tokyo, Japan
SOURCE: Plant and Cell Physiology, (2005) Vol. 46, No. Suppl. S, pp. S148.
Meeting Info.: 46th Annual Meeting of the Japanese-Society-of-Plant-Physiologists. Niigata, JAPAN. March 24 -26, 2005. Japanese Soc Plant Physiologists.
CODEN: PCPHA5. ISSN: 0032-0781.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 16 Nov 2005
Last Updated on STN: 16 Nov 2005

L5 ANSWER 10 OF 12 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-14562 BIOTECHDS
TITLE: New modified green fluorescent protein, useful as a reporter in expression studies;
modified fusion protein prepared by directed evolution

useful as a reporter gene protein

AUTHOR: WALDO G S
PATENT ASSIGNEE: WALDO G S
PATENT INFO: US 2004078148 22 Apr 2004
APPLICATION INFO: US 2003-423688 24 Apr 2003
PRIORITY INFO: US 2003-423688 24 Apr 2003; US 2002-132067 24 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-340059 [31]

AB DERWENT ABSTRACT:

NOVELTY - A green fluorescent protein that comprises at least 80% identity to a fully defined sequence of 56 amino acids (SEQ ID NO:1); (b) at least one amino acid substitution selected from e.g., a substitution at position 30 that is an arginine or a conservative variant of arginine; and (c) measurable fluorescence activity.

DETAILED DESCRIPTION - A green fluorescent protein that comprises at least 80% identity to a fully defined sequence of 56 amino acids (SEQ ID NO:1); (b) at least one amino acid substitution selected from the group consisting of a substitution at position 30 that is an arginine or a conservative variant of arginine; a substitution at position 39 that is an asparagine or a conservative variant of asparagine; a substitution at position 105 that is a threonine or a conservative variant of threonine; a substitution at position 171 that is a valine; and a substitution at position 206 that is a valine; where the positions are determined in alignment for maximal correspondence with SEQ ID NO:1; and (c) measurable fluorescence activity. INDEPENDENT CLAIMS are also included for the following: (1) directed-evolution (M1) of generating enhanced folding variant of target polypeptide, comprising mutating a polynucleotide encoding polypeptide of interest to generate a library of mutated polynucleotides, linking mutated polynucleotides to polynucleotide encoding folding interference domain to form fusion protein (FP) constructs, expressing FP, and selecting FP that display optimal folding activity in relation to FP comprising wild-type polypeptide and folding interference domain, thus identifying polypeptide with enhanced folding activity.(2) an enhanced folding variant (I) of a fluorescent protein generated by (M1); (3) an enhanced folding variant (II) of a chromophoric protein generated by (M1); (4) enhancing (M2) folding of a polypeptide comprising multiple domains, involves joining a first domain of the polypeptide to a poorly folding domain, to form a fusion protein, mutating the first domain, detecting an increase in the amount of activity generated by a first mutated fusion protein in comparison to a fusion protein comprising a wild-type first domain and the poorly folding polypeptide domain, thus identifying a first domain with enhanced folding, joining a second domain of the polypeptide to the first mutated fusion protein to form a second fusion protein, mutating the second domain, and detecting an increase in the amount of activity generated by a second mutated fusion protein in comparison to a fusion protein comprising the wild-type second domain and the first mutated fusion protein, thus identifying a target polypeptide with multiple domains that have enhanced folding; (5) an isolated nucleic acid (IV) encoding a green fluorescent protein that has at least 80% identity to (S1), at least one amino acid substitution as described above, and measurable fluorescent activity; (6) an expression vector (V) comprising (IV); and (7) a host cell comprising (V).

BIOTECHNOLOGY - Preferred Methods: (M1) further involves carrying out one or more subsequent rounds of directed evolution on a fusion protein construct encoding an initially selected fusion protein that display optimal folding activity, and selecting fusion proteins from it with optimal folding activity relative to the folding activity of the initially selected fusion protein, thus identifying polypeptides with further enhanced folding activity. The folding activity is determined by measuring folding kinetics or by measuring resistance to denaturation by urea. The folding activity of the fusion protein is determined by measuring a biological activity of the target polypeptide. The target

polypeptide is a fluorescent protein and the biological activity is fluorescence. The target polypeptide is a chromophoric protein and the biological activity is color. The fluorescent protein is chosen from Aequorea victoria green fluorescent protein and a fluorescent protein having a structure with a root mean square deviation of less than 5Angstrom from the 11- stranded beta-barrel component of the A.victoria Green fluorescent protein structure MMDB Id: 5742. Preferred Variant: In (I), the fluorescent protein is A.victoria green fluorescent protein, red fluorescent protein from a Discosoma species, Anthosoan fluorescent protein, Trachyphyllia geoffroyi , or Anemonia sulcata. In (II), the fluorescent protein has a fully defined amino acid sequence of 182 amino acids (S2), or 168 amino acids (S3). The target polypeptide is a reporter polypeptide. The fusion protein further comprises a reporter molecule that has the reporter activity. The reporter activity is chosen from fluorescent signal or antibiotic resistance. The poorly folding domain is a ferritin domain. The fusion protein comprises a target polypeptide linked to the poorly folding domain by a linker. The reporter molecule is linked to the fusion protein. The folding interference domain is inserted into permissive sites of the target polypeptide. The target polypeptide is green fluorescent protein that has at least 80% identity when aligned for maximum correspondence to (S2) or to a fully defined sequence of 180 amino acids (S4), and has fluorescent activity. Preferred Protein: (III) further comprises a phenylalanine substitution at position 145. The amino acid substitution is chosen from arginine substitution at position 30, an asparagine substitution position 39, threonine substitution at position 105, valine substitution at position 171, and a valine substitution at position 206. The substitution is arginine at position 30, asparagine at position 39, threonine at position 105, phenylalanine at position 145, valine at position 171, or valine at position 206. The green fluorescent protein comprises any two, three, four, or five substitutions as described in (III). The five substitutions are an arginine at position 30, asparagine at position 39, threonine at position 105, valine at position 171 and valine at position 206. The green fluorescent protein further comprises a sixth substitution that is a phenylalanine at position 145. (III) further comprises a mutation chosen from Phe99Ser, Met153Thr and Val163Ala. (III) is cyclized. Preferred Nucleic Acid: (IV) encodes a green fluorescent protein having at least one amino acid substitution chosen from arginine substitution at position 30, asparagine substitution at position 39, threonine substitution at position 105, phenylalanine substitution at position 145, valine substitution at position 171, and a valine substitution at position 206.

USE - (I) and (II) are useful as reporter proteins to express the report level of a protein. (M1) is useful for directed-evolution of generating enhanced folding variant of target polypeptide (claimed). (M1) is useful for improving folding and solubility of a target protein.

ADVANTAGE - (I) provides new and more stable scaffolds for the creation of new GFP variants.

EXAMPLE - To test the effect of the superfolder mutations, 6 single-point mutants of cycle-3 redshift were engineered by PCR. Each mutant incorporated one of the 6 mutations found the superholder green fluorescent protein (GFP) variant. These were cloned into a pET vector as C-terminal fusions with poorly-folded bullfrog redcell ferritin. Rapid protein-folding assay using green fluorescent protein was carried out. Overnight cultures in Luria-Bertani (LB) media containing kanamycin (35 g/ml) were diluted 100-fold and grown for 2 hours at 37degreesC. Proteins were expressed for 4 hours by adding isopropyl-D-thiogalactopyranoside (IPTG) to 1 mM in 3 ml cultures of LB media at either 37degreesC or 27degreesC in Escherichia coli BL21(DE3) as C-terminal fusions with poorly-folded bull frog red cell H-subunit ferritin. Cycle-3 redshift and superfolder were cloned and expressed similarly as controls, both with and without the N-terminal ferritin. The fluorescence (488 nm ex/520 nm em) and absorbance (600 nm) were measured for each culture using a BioTek FL-600 plate reader. (46 pages)

L5 ANSWER 11 OF 12 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2006:1252707 HCAPLUS
DOCUMENT NUMBER: 146:23029
TITLE: Optical microscopy with phototransformable optical labels
INVENTOR(S): Hess, Harald F.; Betzig, Robert E.
PATENT ASSIGNEE(S): Hess, Harald, F., USA; Betzig, Robert, E.
SOURCE: PCT Int. Appl., 75 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2006127692	A2	20061130	WO 2006-US19887	20060523
WO 2006127692	A3	20070426		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AP, EA, EP, OA

PRIORITY APPLN. INFO.: US 2005-683337P P 20050523
US 2006-780968P P 20060310

AB First activation radiation is provided to a sample that includes phototransformable optical labels ("PTOLs") to activate a first subset of the PTOLs in the sample. First excitation radiation is provided to the first subset of PTOLs in the sample to excite at least some of the activated PTOLs, and radiation emitted from activated and excited PTOLs within the first subset of PTOLs is detecting with imaging optics. The first activation radiation is controlled such that the mean volume per activated PTOLs in the first subset is greater than or approx. equal to a diffraction-limited resolution volume ("DLRV") of the imaging optics.

L5 ANSWER 12 OF 12 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2003:886090 HCAPLUS
DOCUMENT NUMBER: 140:107188
TITLE: Photo-induced peptide cleavage in the green -to-red conversion of a fluorescent protein
AUTHOR(S): Mizuno, Hideaki; Mal, Tapas Kumar; Tong, Kit I.; Ando, Ryoko; Furuta, Toshiaki; Ikura, Mitsuhiko; Miyawaki, Atsushi
CORPORATE SOURCE: Laboratory for Cell Function Dynamics, Advanced Technology Development Group, Brain Science Institute, The Institute of Physical and Chemical Science (RIKEN), Wako-city, 351-0198, Japan
SOURCE: Molecular Cell (2003), 12(4), 1051-1058
CODEN: MOCEFL; ISSN: 1097-2765
PUBLISHER: Cell Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Green fluorescent protein from the jellyfish (Aequorea GFP) and GFP-like proteins from coral species encode light-absorbing chromophores within their protein sequences. A coral fluorescent protein, Kaede, contains a tripeptide, His62-Tyr63-Gly64, which acts as a green

chromophore that is photoconverted to red. Here, the authors present the structural basis for the green-to-red photoconversion. As in Aequorea GFP, a chromophore, 4-(p-hydroxybenzylidene)-5-imidazolinone, derived from the tripeptide mediates green fluorescence in Kaede. UV irradiation causes an unconventional cleavage within Kaede protein between the amide nitrogen and the α carbon (Cα) at His62 via a formal β-elimination reaction, which requires the whole, intact protein for its catalysis. The subsequent formation of a double bond between His62-Cα and -Cβ extends the π-conjugation to the imidazole ring of His62, creating a new red-emitting chromophore, 2-[(1E)-2-(5-imidazolyl)ethenyl]-4-(p-hydroxybenzylidene)-5-imidazolinone. The present study not only reveals diversity in the chemical structure of fluorescent proteins but also adds a new dimension to posttranslational modification mechanisms.

REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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E8           10     KARASAWA SADAJI/AU
E9            7     KARASAWA SADATSUGU/AU
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E4            1      MIZUNO H B/AU
E5           76      MIZUNO HAJIME/AU
E6            1      MIZUNO HAKUJI/AU
E7            1      MIZUNO HALSUHIKO/AU
E8            9      MIZUNO HANA/AU
E9            6      MIZUNO HARUHIKO/AU
E10           2      MIZUNO HARUKI/AU
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E12           9      MIZUNO HARUMITSU/AU

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L9         2103 "MIZUNO H"/AU

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     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 10:21:01 ON 05 OCT 2007
L1          1378 S FLUOROSCEN?
L2            38 S TRACHYPHYLLIA (W) GEOFFROYI
L3             0 S L1 AND L2
L4            22 DUP REM L2 (16 DUPLICATES REMOVED)
L5            12 S L4 AND GREEN
                 E MIYAWAKI A/AU
L6           515 S E3
                 E ANDO R/AU
L7           594 S E3
                 E KARASAWA S/AU
L8            43 S E12
                 E MIZUNO H/AU
L9          2103 S E3

=> s l6 or l7 or l8 or l9
L10        3191 L6 OR L7 OR L8 OR L9

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L11          14 L2 AND L10

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PROCESSING COMPLETED FOR L11
L12           6 DUP REM L11 (8 DUPLICATES REMOVED)

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L12  ANSWER 1 OF 6  EMBASE  COPYRIGHT (c) 2007 Elsevier B.V. All rights
     reserved on STN
ACCESSION NUMBER:    2007438285  EMBASE
TITLE:               Crystallographic Evidence for Water-assisted Photo-induced
                     Peptide Cleavage in the Stony Coral Fluorescent Protein
                     Kaede.
AUTHOR:              Hayashi I.; Mizuno H.; Tong K.I.; Furuta T.;
                     Tanaka F.; Yoshimura M.; Miyawaki A.; Ikura M.
CORPORATE SOURCE:    M. Ikura, Division of Signaling Biology, Ontario Cancer
                     Institute, Department of Medical Biophysics, 101 College
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St, Toronto, Ont. M5G 1L7, Canada.
mikura@uhnres.utoronto.ca
SOURCE: Journal of Molecular Biology, (28 Sep 2007) Vol. 372, No. 4, pp. 918-926.
Refs: 34
ISSN: 0022-2836 CODEN: JMOBAK
PUBLISHER IDENT.: S 0022-2836(07)00831-5
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical and Experimental Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 24 Sep 2007
Last Updated on STN: 24 Sep 2007

AB A coral fluorescent protein from Trachyphyllia geoffroyi , Kaede, possesses a tripeptide of His62-Tyr63-Gly64, which forms a chromophore with green fluorescence. This chromophore's fluorescence turns red following UV light irradiation. We have previously shown that such photoconversion is achieved by a formal β-elimination reaction, which results in a cleavage of the peptide bond found between the amide nitrogen and the α-carbon at His62. However, the stereochemical arrangement of the chromophore and the precise structural basis for this reaction mechanism previously remained unknown. Here, we report the crystal structures of the green and red form of Kaede at 1.4 A and 1.6 A resolutions, respectively. Our structures depict the cleaved peptide bond in the red form. The chromophore conformations both in the green and red forms are similar, except a well-defined water molecule in the proximity of the His62 imidazole ring in the green form. We propose a molecular mechanism for green-to-red photoconversion, which is assisted by the water molecule.

L12 ANSWER 2 OF 6 MEDLINE on STN
ACCESSION NUMBER: 2006452697 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16753144
TITLE: Dynamic behavior of individual cells in developing organotypic brain slices revealed by the photoconvertable protein Kaede.
AUTHOR: Mutoh T; Miyata T; Kashiwagi S; Miyawaki A; Ogawa M
CORPORATE SOURCE: Laboratory for Cell Culture Development, Advanced Technology Development Center, Brain Science Institute, Riken Saitama, Japan.. tmuto@brain.riken.jp
SOURCE: Experimental neurology, (2006 Aug) Vol. 200, No. 2, pp. 430-7. Electronic Publication: 2006-06-06.
Journal code: 0370712. ISSN: 0014-4886.
PUB. COUNTRY: United States
DOCUMENT TYPE: (COMPARATIVE STUDY)
(IN VITRO)
Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200609
ENTRY DATE: Entered STN: 1 Aug 2006
Last Updated on STN: 22 Sep 2006
Entered Medline: 21 Sep 2006

AB In recent years, advances in optical imaging methods have facilitated the visualization of events in the developing cortex. In particular, the introduction of DNA encoding fluorescent protein into cells of the embryonic brain allows the visualization of progenitor cells; slice preparations of the cortex then allow the monitoring of the behavior of transfected cells in the context of the living cerebral wall by time-lapse microscopy. Such approaches have provided substantial information about the patterns of neuronal migration. However, as these techniques label

large numbers of cells in the ventricular zone (VZ), it is difficult to follow individual cell shape changes or cell behaviors within the VZ, where neuron production and initial migration take place. Here, we report a unique method using the photoconvertable fluorescent protein Kaede, which emits green fluorescence and shifts to emitting red fluorescence upon radiation with UV. Using this method, we were able to follow the behavior of a particular pair of daughter cells among neighboring Kaede-positive cells in the SVZ of mouse brain slices. The spindle shape progenitor divided into two multipolar-shaped daughter cells. The cell-cell borders of daughter cells were clearly visualized, and easily describe the position and distance between two or more cells. The photoconvertable property of Kaede offers a powerful cell marking tool to identify the precise morphology and migratory behaviors of individual cells within living cortical slices.

L12 ANSWER 3 OF 6 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:569901 BIOSIS
DOCUMENT NUMBER: PREV200600582693
TITLE: Cloning and characterization of novel fluorescent proteins from Anthozoan animals and their applications to cell biological research.
AUTHOR(S): Karasawa, Satoshi; Miyawaki, Atsushi
SOURCE: Zoological Science (Tokyo), (DEC 2005) Vol. 22, No. 12, pp. 1417-1418.
Meeting Info.: 76th Annual Meeting of the Zoological-Society-of-Japan. Tsukuba, JAPAN. October 06 -08, 2005. Zool Soc Japan.
CODEN: ZOSCEX. ISSN: 0289-0003.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 1 Nov 2006
Last Updated on STN: 1 Nov 2006

L12 ANSWER 4 OF 6 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2005110192 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15731765
TITLE: Semi-rational engineering of a coral fluorescent protein into an efficient highlighter.
AUTHOR: Tsutsui Hidekazu; Karasawa Satoshi; Shimizu Hideaki; Nukina Nobuyuki; Miyawaki Atsushi
CORPORATE SOURCE: Laboratory for Cell Function Dynamics, Brain Science Institute, RIKEN, 2-1 Hirosawa, Wako-city, Saitama 351-0198, Japan.
SOURCE: EMBO reports, (2005 Mar) Vol. 6, No. 3, pp. 233-8.
Journal code: 100963049. ISSN: 1469-221X.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200508
ENTRY DATE: Entered STN: 3 Mar 2005
Last Updated on STN: 9 Aug 2005
Entered Medline: 8 Aug 2005

AB Kaede is a natural photoconvertible fluorescent protein found in the coral Trachyphyllia geoffroyi. It contains a tripeptide, His 62-Tyr 63-Gly 64, which acts as a green chromophore that is photoconvertible to red following (ultra-) violet irradiation. Here, we report the molecular cloning and crystal structure determination of a new fluorescent protein, KikG, from the coral Favia favus, and its in vitro evolution conferring green-to-red photoconvertibility. Substitution of the His 62-Tyr 63-Gly 64 sequence into the native protein provided only negligible photoconversion. On the basis of the crystal structure,

semi-rational mutagenesis of the amino acids surrounding the chromophore was performed, leading to the generation of an efficient highlighter, KikGR. Within mammalian cells, KikGR is more efficiently photoconverted and is several-fold brighter in both the green and red states than Kaede. In addition, KikGR was successfully photoconverted using two-photon excitation microscopy at 760 nm, ensuring optical cell labelling with better spatial discrimination in thick and highly scattering tissues.

L12 ANSWER 5 OF 6 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
DUPLICATE 2
ACCESSION NUMBER: 2004-10115 BIOTECHDS
TITLE: Chromoprotein from Anthopleura inornata and fluoroproteins from Trachyphyllia geoffroyi and Scolymia vitiensis for optical marking of cells and organs; involving vector-mediated gene transfer and expression in host cell for use in cell and organ optical marking
AUTHOR: MIYAWAKI A; ANDO R; KARASAWA S; MIZUNO H
PATENT ASSIGNEE: RIKEN KK; MEDICAL and BIOLOGICAL LAB CO LTD
PATENT INFO: WO 2004018671 4 Mar 2004
APPLICATION INFO: WO 2003-JP10628 22 Aug 2003
PRIORITY INFO: JP 2002-280118 26 Sep 2002; JP 2002-243337 23 Aug 2002
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 2004-226849 [21]
AB DERWENT ABSTRACT:
NOVELTY - Chromoprotein derived from Anthopleura inornata and chromoproteins derived from it by addition, deletion and/or substitution of one or more amino acid residues, are new.

DETAILED DESCRIPTION - Chromoprotein derived from the coral Anthopleura inornata and chromoproteins derived from it by addition, deletion and/or substitution of one or more amino acid residues, are new. The chromoprotein has a working pH range of 5-10. The amino acid sequences of two forms of the chromoprotein are given having respectively an absorption maximum at 605nm (molar absorption 47550) and at 553nm (molar absorption 25300). INDEPENDENT CLAIMS are also given for: (1) fluoroprotein derived from the coral Trachyphyllia geoffroyi and fluoroproteins derived from it by addition, deletion and/or substitution of one or more amino acid residues. The fluoroprotein has absorption maxima at 508nm (molar absorption 98800) and 572nm (molar absorption 60400) and emission maxima at 518nm and 581nm with quantum yields of 0.80 and 0.33 respectively. The pKa is 5.7. (2) fluoroprotein derived from the coral Scolymia vitiensis and fluoroproteins derived from it by addition, deletion and/or substitution of one or more amino acid residues. The fluoroprotein has absorption maxima at 508nm (molar absorption 102250) and 578nm (molar absorption 76950) and emission maxima at 518nm and 588nm with quantum yields of 0.43 and 0.51 respectively. The pKa is 5.8 (508nm) or 6.5 (578nm); (3) DNA encoding the chromoprotein and fluoroproteins; (4) expression vectors containing this DNA; (5) hosts transformed by the vectors; (6) fusion proteins containing the chromoprotein or fluoroproteins together with another protein; (7) analysis of a biologically active protein and its function using the fluorescence resonance energy transition (FRET) method with the chromoprotein from Anthopleura inornata as acceptor; (8) analysis of function of a protein within the cell by expression of a fusion protein of the protein with the coral-derived chromoprotein or fluoroproteins within the cell; and (9) kits for these analysis methods.

USE - Optical marking of cells and organs and analysis of biologically active proteins and their function.

EXAMPLE - Total RNA is isolated from the coral Anthopleura inornata. The RNA is used to synthesize cDNA using a Ready-to-Go First Strand cDNA Synthesis Kit (Amersham-Pharmacia). The cDNA is amplified by polymerase chain reaction (PCR) using primer 5'-CCCGGATCCGACCATGGCTACCTTGGTTAAAGA-3' and oligo-dT primer, to yield a 1100bp fragment which is inserted into

pRSET vector (Invitrogen) and used to transform Escherichia coli JM109-DE3. The transformant is cultured and the protein purified on Ni-Agarose gel (Qiagen) to give the chromoprotein (Be-G). The protein shows absorption maxima at 277nm and 605nm. (118 pages)

L12 ANSWER 6 OF 6 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN DUPLICATE 3

ACCESSION NUMBER: 2002360292 EMBASE
TITLE: An optical marker based on the UV-induced green-to-red photoconversion of a fluorescent protein.
AUTHOR: Ando R.; Hama H.; Yamamoto-Hino M.; Mizuno H.; Miyawaki A.
CORPORATE SOURCE: A. Miyawaki, Laboratory for Cell Function, Adv. Technology Development Center, Brain Science Institute, 2-1 Hirosawa, Wako-city, Saitama 351-0198, Japan. matsushi@brain.riken.go.jp
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1 Oct 2002) Vol. 99, No. 20, pp. 12651-12656.
Refs: 19
ISSN: 0027-8424 CODEN: PNASA6
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical and Experimental Biochemistry
008 Neurology and Neurosurgery
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 24 Oct 2002
Last Updated on STN: 24 Oct 2002

AB We have cloned a gene encoding a fluorescent protein from a stony coral, Trachyphyllia geoffroyi, which emits green, yellow, and red light. The protein, named Kaede, includes a tripeptide, His-Tyr-Gly, that acts as a green chromophore that can be converted to red. The red fluorescence is comparable in intensity to the green and is stable under usual aerobic conditions. We found that the green-red conversion is highly sensitive to irradiation with UV or violet light (350-400 nm), which excites the protonated form of the chromophore. The excitation lights used to elicit red and green fluorescence do not induce photoconversion. Under a conventional epifluorescence microscope, Kaede protein expressed in HeLa cells turned red in a graded fashion in response to UV illumination; maximal illumination resulted in a 2,000-fold increase in the ratio of red-to-green signal. These color-changing properties provide a simple and powerful technique for regional optical marking. A focused UV pulse creates an instantaneous plane source of red Kaede within the cytosol. The red spot spreads rapidly throughout the cytosol, indicating its free diffusibility in the compartment. The extensive diffusion allows us to delineate a single neuron in a dense culture, where processes originating from many different somata are present. Illumination of a focused UV pulse onto the soma of a Kaede-expressing neuron resulted in filling of all processes with red fluorescence, allowing visualization of contact sites between the red and green neurons of interest.

=> d his

(FILE 'HOME' ENTERED AT 10:20:28 ON 05 OCT 2007)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:21:01 ON 05 OCT 2007

L1 1378 S FLUOROSCEN?
L2 38 S TRACHYPHYLLIA (W) GEOFFROYI
L3 0 S L1 AND L2
L4 22 DUP REM L2 (16 DUPLICATES REMOVED)

L5	12	S	L4 AND GREEN
		E	MIYAWAKI A/AU
L6	515	S	E3
		E	ANDO R/AU
L7	594	S	E3
		E	KARASAWA S/AU
L8	43	S	E12
		E	MIZUNO H/AU
L9	2103	S	E3
L10	3191	S	L6 OR L7 OR L8 OR L9
L11	14	S	L2 AND L10
L12	6	DUP REM L11 (8 DUPLICATES REMOVED)	

	L #	Hits	Search Text
1	L1	8	trachyphyllia adj geoffroyi
2	L2	8	green same l1
3	L3	586	chromoprotein$2
4	L4	8	l1 same l3
5	L5	36968	MIYAWAKI ANDO KARASAWA MIZUNO
6	L6	8	l1 and l5

	Document ID	Kind Codes	Source	Issue Date	Pages	Title
1	US 20060257942 A1		US-PGPUB	20061116	63	Protein subcellular localization assays using split fluorescent proteins
2	US 20060257887 A1		US-PGPUB	20061116	83	Protein -protein interaction detection system using fluorescent protein microdomains
3	US 20060252063 A1		US-PGPUB	20061109	75	Circular permutant GFP insertion folding reporters
4	US 20060160990 A1		US-PGPUB	20060720	53	Fluorescent protein and chromoprotein
5	US 20050221343 A1		US-PGPUB	20051006	80	Self-assembling split-fluorescent protein systems
6	US 20050142637 A1		US-PGPUB	20050630	17	Long-wavelength FPs
7	US 20040078148 A1		US-PGPUB	20040422	46	Directed evolution methods for improving polypeptide folding and solubility and superfolder fluorescent proteins generated thereby
8	US 7271241 B2		USPAT	20070918	43	Directed evolution methods for improving polypeptide folding and solubility and superfolder fluorescent proteins generated thereby

	Document ID	Kind Codes	Source	Issue Date	Pages	Title
1	US 20060257942 A1		US-PGPUB	20061116	63	Protein subcellular localization assays using split fluorescent proteins
2	US 20060257887 A1		US-PGPUB	20061116	83	Protein -protein interaction detection system using fluorescent protein microdomains
3	US 20060252063 A1		US-PGPUB	20061109	75	Circular permutant GFP insertion folding reporters
4	US 20060160990 A1		US-PGPUB	20060720	53	Fluorescent protein and chromoprotein
5	US 20050221343 A1		US-PGPUB	20051006	80	Self-assembling split-fluorescent protein systems
6	US 20050142637 A1		US-PGPUB	20050630	17	Long-wavelength FPs
7	US 20040078148 A1		US-PGPUB	20040422	46	Directed evolution methods for improving polypeptide folding and solubility and superfolder fluorescent proteins generated thereby
8	US 7271241 B2		USPAT	20070918	43	Directed evolution methods for improving polypeptide folding and solubility and superfolder fluorescent proteins generated thereby